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Report I

Selection of a Tissue Culture, Most Sensitive to the Action of Staphylococcus Toxin

TRANSLATION NO. 1422

June 1965

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STUDY OF THE CYTOTOXIC EFFECT OF STAPHYLOCOCCUS TOXIN ON TISSUE CULTURE

Report I

Selection of a Tissue Culture, Most Sensitive to the Action of Staphylococcus Toxin

[Following is the translation of an article by Ye. I. Belyayev, Gorkovskiy Institute of Epidemiology and Microbiology, appearing in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology Epidemiology and Immunobiology), No. 10, 1964, pages 32-37. It was submitted on 23 Nov 1963. Translation performed by Sp/7 Charles T. Ostertag Jr.]

The pathogenicity of staphylococci is determined by the Chempen signs: Formation of golden pigment, fermentation of mannite, plasma coagulation and hemolysis. These signs are unstable and only taken together can they serve to judge the degree of pathogenicity of a microbe. Attempts are also known to judge the pathogenicity of staphylococci based on their other properties: Phosphatase activity (Pal et al: Adatov; Lebedeva), splitting of urea and the formation of lecithinase (Chistovich), and effect on animals (Gebhardt), but these features are also variable and taken separately do not permit a judgment on the pathogenicity of the staphylococcal strain under study. In connection with what has been indicated, attempts were undertaken to use a tissue culture for studying the properties of staphylococci and staphylococcal toxins. Andre et al., Goldschneider, and Lawrence et al. established the evident cytotoxic effect of staphylococcal toxin on tissue culture obtained from the various organs of man and animals.

The aim of the present work was a study of the effect of staphylococcal toxins on various cell cultures. In the experiments 11 types of cell cultures of various ages were tested. Of these, 3 were primarily trypsinized -- fibroblasts of a human embryo, chick embryo, and the kidneys of a human embryo, and 8 were transplanted strains -- human amniotic cells (stable FL strain), human liver tissue (Chang strain), Detroit-6, HeLa, monkey kidneys, guinea pig kidneys, and a mixed strain from transplanted cultures (Leningrad-a).

The tissues were prepared by the generally accepted method in the Institutes tissue culture laboratory. For infection we used both whole and diluted, with physiological solution, staphylococcal toxins from the Institute of Epidemiology and Microbiology, AMN, USSR (with various

preparation periods).

For testing the cytotoxic effect, 14 test tubes of various types of tissue culture were taken for each series of toxin. Under sterile conditions the nutrient medium was poured off of all the test tubes with the tissue. The tissue was infected by placing in each test tube 0.2 ml of whole or diluted toxin (2 test tubes were taken for each dilution); 4 test tubes remained as control (2 were not infected and in 2 instead of toxin a physiological solution was added). After being in contact for one hour at room temperature, 1 ml of nutrient medium was added to each test tube and they were placed in an incubator at 37°. The results were considered under the low magnification of a microscope after 24, 48, 72, 96 and 120 hours.

The degree of the cytotoxic effect was evaluated by the following method: + = degeneration of an insignificant number of cells, ++ = degenerative changes in no less than half the cells, +++ = 75% of the cells affected, and ++++ = complete degeneration or slipping of the entire cellular layer. Each test was repeated five times.

Table 1 presents the results of the effect of undiluted toxin series No. 314 on tissue culture of various ages. It turned out that the sensitivity of various tissues to staphylococcal toxin is not the same. The earliest and clearest degeneration was noted in the 24-hour fibroblasts of human embryos and chick embryos and also the Chang strain, in which already after 24 hours the cells were completely degenerated. In these same tissues of a 2-3 day growth, and also in tissues of human amnion, the liver tissue of a human and monkey embryo, and the kidneys of a guinea pig of a 3-4 day growth, the complete degeneration set in after 48 hours. Guinea pig kidneys were an exception: In 4-day cultures of this tissue the complete degeneration set in after 48 hours, and in a 3-day -- after 72 hours. For the transplanted strains -- Hep-2, HeLa, Detroit-6, and the Leningrad-a, both the 2- and the 3-day growth turned out to be more resistant to the effect of staphylococcal toxin -- complete degeneration of these tissues set in only after 72 hours and later. Attempts to capture earlier changes in the tissue cultures -- after 3, 6 and 12 hours, were not successful -- the cytotoxic effect in the first hours following the administration of toxin is variable. It must also be noted that the majority of tissues of a 3-day growth reacted more weakly to the effect of toxin than the 2-day.

Table 2 presents the results of the effect of whole toxins of series No. 314, 257 and 188 with various periods of preparation. It testified that the cytotoxic effect on tissue cultures depended on the antiquity of preparing the toxin. Thus, toxin of series No. 188, prepared 3 years ago, exerted the weakest cytotoxic effect. Tests with diluted

toxins were analogous: Toxins of series No. 314 and 257 in a dilution of 1:10 caused the complete degeneration of 24-hour fibroblasts of human embryo in 24 hours, and toxin from series No. 188 in this same period caused only an initial degeneration of cells. This is testified to by the data in table 3. Toxins diluted by 100 times and more did not exert a cytotoxic effect on tissue cultures.

The specificity of the effect of staphylococcal toxin on tissue culture was confirmed by tests of neutralization with antistaphylococcal horse serum, prepared at the Institute of Epidemiology and Microbiology, AMN, USSR, and containing 110 units (series No. 1) in 1 ml. The neutralization tests were conducted in the following manner: The toxin was mixed with whole serum and serum that had been diluted by 10 and 100 times in even volumes, the mixture was incubated at room temperature for one hour, and then 0.2 ml of the mixture was added to 24-hour cultures of human embryo fibroblasts according to the above described method. The control was tissue, infected only with toxin, which caused the complete degeneration already in 24 hours, while at the same time in the test tubes with a tissue culture, infected with a mixture of toxin with serum (even if the serum was diluted by 100 times) degeneration was absent for 5 days.

Conclusions

1. Staphylococcal toxins exerted a clearly expressed specific cytotoxic effect on tissue cultures of a various origin.
2. A 24-hour culture of human embryo fibroblasts was the most sensitive to the effect of toxin.
3. The power of the toxin depended on the period of its preparation: Freshly prepared toxins exerted the most expressed cytotoxic effect.

Literature

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Table 1

Sensitivity of various types of tissue culture to the effect of staphylococcal toxin series No 314, prepared on 14 Sep 1962.

Type of tissue culture	Degree of cytotoxic effect after various intervals of time (in hours) on cultures of different ages														
	24-hour					48-hour					72-hour				
	24	48	72	96	120	24	48	72	96	120	24	48	72	96	120
Human embryo fibroblasts	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	+++	++++	++++	++++	++++
Chick embryo fibroblasts	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	+++	++++	++++	++++	++++
Chang						++++	++++	++++	++++	++++	+++	++++	++++	++++	++++
Human amnion.....						+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Guinea pig kidney.....						++	++++	++++	++++	++++	+	+++	++++	++++	++++
Monkey kidney.....						+	++++	++++	++++	++++	+	++++	++++	++++	++++
Human embryo kidney.....						+	++++	++++	++++	++++	+	++++	++++	++++	++++
Hep-2.....						+	+++	++++	++++	++++	+	++	++++	++++	++++
Hela.....						+	++	++++	++++	++++	+	+++	++++	++++	++++
Detroit-6..						+	+++	++++	++++	++++	+	++	++++	++++	++++
Leningrad-a						+	+++	++++	++++	++++	+	++++	++++	++++	++++

Degenerative changes of cell cultures following their infection with various series of staphylococcal toxins

Table 2

Type of tissue culture	Level of cytotoxic effect after various intervals of time (in hours)														
	Series No 314 of 14 Sep 62					Series No 257 of 3 Mar 61					Series No 188 of 12 Jan 60				
	24	48	72	96	120	24	48	72	96	120	24	48	72	96	120
Human embryo fibroblasts (24 hr. culture).....	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++
Chick embryo fibroblasts (24 hr. culture).....	++++	++++	++++	++++	++++										
Chang.....	++++	++++	++++	++++	++++										
Human embryo fibroblasts (2-3 day culture).....	+++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++	+++	+++	+++	+++
Chick embryo fibroblasts (2-3 day culture).....	+++	++++	++++	++++	++++										
Human amnion.....	+++	++++	++++	++++	++++	+	++++	++++	++++	++++	+	++++	++++	++++	++++
Guinea pig kidney	++	++++	++++	++++	++++	-	+++	++++	++++	++++	-	+	+++	+++	+++
Monkey kidney.....	+	++++	++++	++++	++++	-	+++	++++	++++	++++	-	+	+++	+++	+++
Human embryo kidney.....	+	++++	++++	++++	++++	++	++++	++++	++++	++++	-	++	+++	+++	+++
Hep-2.....	+	+++	++++	++++	++++	+	+++	++++	++++	++++	-	+	+++	+++	+++
Hela.....	+	++	++++	++++	++++	-	+++	++++	++++	++++	-	+	+++	+++	+++
Detroit-6.....	+	+++	++++	++++	++++	+	+++	++++	++++	++++	-	-	++	+++	+++
Leningrad-a.....	+	+++	++++	++++	++++	-	+	+++	++++	++++	-	-	+	+++	+++

Sensitivity of tissue cultures to the effect of various series of staphylococcal toxins, diluted to 1:10

Table 3

Type of tissue culture	Level of cytotoxic effect after various intervals of time (in hours)														
	Series No 314 of 14 Sep 62					Series No 257 of 3 Mar 61					Series No 188 of 12 Jan 60				
	24	48	72	96	120	24	48	72	96	120	24	48	72	96	120
Human embryo fibroblasts (24 hr. culture).....	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	+	+	+	+	+
Chick embryo fibroblasts (24 hr. culture).....	+++	+++	+++	+++	++										
Chang.....	+	++	+++	+++	+++										
Human embryo fibroblasts (2-3 day culture).....	++	++	+++	+++	++++	+	+	++	++	+++	-	-	-	+	+
Chick embryo fibroblasts (2-3 day culture).....	++	++	+++	+++	++++										
Human amnion.....	-	-	+	-	+	-	-	+	++	+++	-	-	+	+	++
Guinea pig kidney.....	+	-	+	+	++	-	-	+	+	++	-	-	-	-	-
Monkey kidney.....	-	-	+	++	++	-	-	+	+	++	-	-	-	+	++
Human embryo kidney.....	+	+	+	++	++	-	-	+	+	++	-	-	-	-	+
Hep-2.....	-	-	-	-	+	-	-	+	+	++	-	-	-	-	+
HeLa.....	-	-	-	-	+	-	-	+	+	++	-	-	-	-	-
Detroit-6.....	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-
Lenington-2.....	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-